REMARKS

The Office action mailed 20 March 2003 has been received and its contents carefully noted. The pending claims were claims 1-9 and 38-52. Claims 45-52 were withdrawn from consideration and claims 1-9 and 38-44 were rejected. By this amendment, claims 1 and 3 have been amended and claims 2 and 45-52 have been canceled. Support may be found in the specification generally. No statutory new matter has been added. Therefore, entry of the claims as amended and reconsideration is respectfully requested.

Information Disclosure Statement

Applicants submit herewith references AB1, AC1, and AD1 and a new PTO form 1449 that provides the information missing in the PTO form 1449 submitted on 3 October 2001. Applicants note that AB1 has 372 pages and have therefore submitted AB1 as a pdf file on a CDROM. If such submission on CDROM is not acceptable, Applicants will print out the file and submit the paper copy upon request.

Specification

The Examiner objected to the specification for various informalities.

Applicants respectfully submit that the specification as amended herein address the informalities noted by the Examiner. Therefore, the objection to the specification should properly be withdrawn.

Rejection under 35 U.S.C. 112, second paragraph

The Examiner rejected claims 1-9 and 38-44 under 35 U.S.C. 112, second paragraph, as being indefinite. Specifically, the Examiner:

1. Questioned whether the phrase "with a sensitivity coefficient of each of substrate for the protein" should be "using a sensitivity coefficient for each substrate and for each protein" in claim 1.
2. Deemed that claim 1 is missing steps as it does not recite how to determine the sensitivity coefficient.
3. Deemed that claim 1 is missing a reaction from which the activity can be monitored.
4. Questioned whether more than one substrate should be employed in claim 1.

5. Deemed that claim 2 lacks antecedent basis for "each substrate".
6. Deemed that claim 3 does not tell how to "extract" each sensitivity coefficient and therefore appears incomplete and unclear.
7. Deemed that the steps of claim 4 are unclear and that the method cannot be understood as recited.

Applicants respectfully submit that the claims as amended obviate the rejection under 35 U.S.C. 112, second paragraph.

Applicants note that the meaning of "sensitivity coefficient" and the methods for obtaining the "sensitivity coefficients" may not be fully appreciated by the Examiner. A sensitivity coefficient is a constant that represents the amount or weight (fraction/percentage) a protein contributes to a reaction with a given substrate. Sensitivity coefficients may be determined in a variety of ways which include determining the reaction rates as a function of the sample concentration at infinite inhibitor concentration, plotting the best fit linear graphs, determining the slopes of the linear graphs and then using simple subtraction to calculate the amount each protein contributes to the reaction.

Example 3 in the specification provides a detailed protocol for determining sensitivity coefficients of AChE and BChE towards ACT, PTC, and BTC. Each titration was fitted to the following equation:

$$V_{obs} = \frac{(Vc - Vr)K_I}{K_I + [I]} + Vr \qquad (1)$$

Since a plurality of proteins may have overlapping specificities for given substrates, all observable signals represent the sum total action of the plurality of proteins and cannot in and of themselves be used for directly measuring the amount of a single protein belonging to the plurality of proteins. Thus, the present invention provides a method for determining the weighted contribution for each protein belonging to a plurality of proteins in their total action on the given substrates. The weighted contributions are the sensitivity coefficients.

The concentration or activity of a given protein belonging to a plurality of proteins for a given substrate may be determined by treating each protein as an algebraic unknown and each observable reaction with the substrate as a result. As long as there is at least as many observables/results as unknowns, then each unknown can be explicatively quantified using any

number of elementary algebraic methods for solving systems/sets of equations such as linear substitution/combinations known in the art. The only requirement is that the sensitivity coefficient for each unknown, i.e. the weight (fraction/percentage) that each unknown contributes to the overall observable reaction for the particular unknown need only be determined once.

The sensitivity coefficients may be obtained without adulterating the sensitivity coefficient sample by any number of methods including physically removing one of the unknowns, which usually alters the sensitivity coefficient sample and thereby changing the intrinsic contributions of each protein (sensitivity coefficients). Thus, a better alternative is to destroy the contribution of a given protein to the overall rate via a selective inhibitor. In this manner, it is possible to remove only the desired activity and leave the others in tact. This is best accomplished via a titration curve (see, for example Figure A below) at a predetermined concentration of the sensitivity coefficient sample.

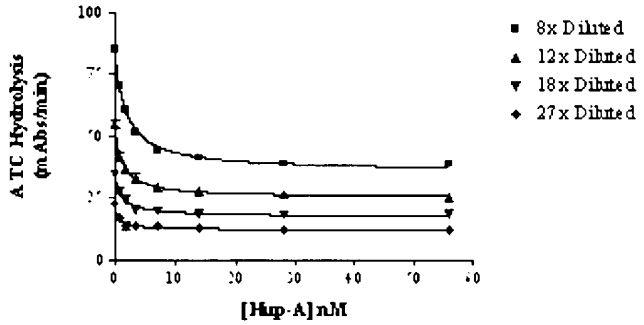

Figure A

So long as the predetermined concentration provides a signal that is interpretable over the entire range of the titration (i.e., 0 to infinite inhibitor concentration), then the asymptote represents the contribution of all other components of the system to the observable. The delta between the control value and the asymptote corresponds to the contribution that the selectively removed unknown contributed to the observable and is the (sensitivity) coefficient for that unknown at that sample concentration. If this is done as a function of sample concentration (Figure A), then the resulting plot displays how the sensitivity coefficients scale with sample concentration (see, for example, Figure B).

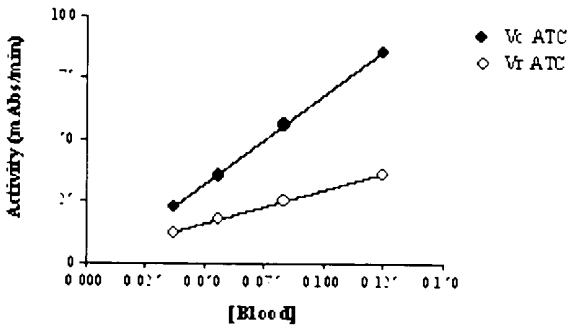

Figure B

Note that one needs to selectively remove each unknown component so that each sensitivity coefficient can be uniquely determined. It is possible to use inferred values based on the total observable minus the uniquely determined sensitivity coefficients to obtain either the background or a remaining unknown's sensitivity coefficient in cases where selective inhibitors are not available. Likewise, a combination of selective inhibitors can be used to destroy activity to determine the sensitivity coefficient (i.e., asymptote) for an unknown for which a selective inhibitor is unknown or unavailable. In any case, the coefficients should be substantially identical.

As previously mentioned, with the sensitivity coefficients in hand, it is possible to use standard algebraic methods to uniquely quantify the concentrations of unknown proteins belonging to a plurality of proteins. For example, the concentrations a first protein, fp, and a second protein, sp, may be determined using the following mathematical equations:

$$x_1[fp] + y_1[sp] = R_1 \qquad \text{Substrate 1}$$
$$x_2[fp] + y_2[sp] = R_2 \qquad \text{Substrate 2}$$
$$x_3[fp] + y_3[sp] = R_3 \qquad \text{Substrate 3}$$

$$[fp]_{1,2} = \frac{\begin{vmatrix} x_1 & R_1 \\ x_2 & R_2 \end{vmatrix}}{\begin{vmatrix} x_1 & y_1 \\ x_2 & y_2 \end{vmatrix}} \quad \text{and} \quad [\text{fp}]_{1,3} = \frac{\begin{vmatrix} x_1 & R_1 \\ x_3 & R_3 \end{vmatrix}}{\begin{vmatrix} x_1 & y_1 \\ x_3 & y_3 \end{vmatrix}} \quad \text{and} \quad [\text{fp}]_{2,3} = \frac{\begin{vmatrix} x_2 & R_2 \\ x_3 & R_3 \end{vmatrix}}{\begin{vmatrix} x_2 & y_2 \\ x_3 & y_3 \end{vmatrix}}$$

$$\therefore mean\,[fp] = \frac{[fp]_{1,2} + [fp]_{1,3} + [fp]_{2,3}}{3}$$

The [sp] is calculated in the same manner as the [fp].

As provided above, the rates of substrate hydrolysis are represented by R1, R2, and R3 and correspond to the turnover of substrate 1, substrate 2, and substrate 3, respectively. The [fp] and [sp] refer to the actual concentrations of fp and sp in the sample. Finally, the coefficients in each equation (i.e., x1, x2, x3, and y1, y2, y3) represent sensitivity coefficients and are the contribution that fp and sp independently contribute to the overall rate of hydrolysis of each substrate (R1, R2, R3). Simultaneously solving these three sets of degenerate equations provides three independent estimates for the concentrations of fp and sp. Therefore, determining the mean value and the standard deviation for these independently derived values provides the concentrations of each protein, fp and sp. Examples of this method are provided in detail in Example 3, entitled "Sensitivity Coefficient Determination, Method 1", Example 4, entitled "Sensitivity Coefficient: Method 2" and may also be visualized in Figures 10A and 10B. See pages 37-42, and the figure legend described on page 14.

Therefore, Applicants respectfully submit that the scope and meaning of "sensitivity coefficient" in the claims is clear and definite and need not be expressed or limited to a mathematical expression. As Applicants have found that the concept of "sensitivity coefficients" has been initially challenging for some of the Applicants' colleagues to understand, Applicants would greatly appreciate an in-person interview be granted prior to any adverse Office action such that prosecution on the merits may be advanced rather than being hindered by semantics and algebraic expressions.

Rejection under 35 U.S.C. 102(b)

The '688 Patent

The Examiner rejected claims 1, 2, 5, 38, and 39 under 35 U.S.C. 102(b) as being anticipated by U.S. Patent No. 5,674,688 ('688 patent). Specifically, the Examiner deemed that the '688 patent discloses a method of assaying structurally related analytes using a panel of reactive reagents to create a reactivity profile which the Examiner deems to be equivalent to "sensitivity coefficients".

Applicants respectfully submit that the "reactivity profile" or "SC profile" of the '688 patent is not the same or equivalent to the "sensitivity coefficients" of the present invention. Most important, the reactivity profile of the '688 patent is a panel of a plurality of specific binding reagents, such as antibodies, in which each specific binding reagent is isolated in a

separate compartment or area. Not all the specific binding reagents will bind a given analyte. See the '688 patent, col. 5, lines 9-28, and col. 7, lines 30-45. Thus, the pattern of binding on the panel such as a 96-well assay plate will produce a characteristic pattern, i.e. reactivity profile. For example, a given analyte will react with the antibodies in wells 1-4, 21, 35, 56, etc.

In contrast, the assay of the present invention does not require a panel to which binding reagents are fixed and then comparing the binding pattern of the fixed panel to a control. Instead, the present invention is based on sensitivity coefficients, which are the weighted contributions that a specific binding reagent reacts with a substrate in the presence of other specific binding reagents that also react with the same substrate. The specific binding reagents (proteins) need not be isolated or separated out from a mixture of a plurality of specific binding reagents. There is no need to have a fixed panel of specific binding agents.

Another important distinction is that the '688 patent only teaches assays of a sample having *a single analyte*, with multiple antibodies to characterize this analyte. In marked contrast, the present invention allows the concentration or activity of a plurality of proteins in one sample to be determined.

Since the "reactivity profile" of the '688 patent is clearly different from the sensitivity coefficients of the present invention and nowhere does the '688 patent teach or disclose use of sensitivity coefficients as taught by the present invention, the '688 patent does not anticipate the claimed invention. Therefore, the rejection under 35 U.S.C. 102(b) should properly be withdrawn.

Chemnitius et al.

The Examiner also rejected claims 1-9, 38, 39, and 41-43 under 35 U.S.C. 102(b) as being anticipated by Chemnitius *et al.* Specifically, the Examiner deemed that Chemnitius *et al.* disclose a differential inhibition method for the quantitative determination of cholinesterase isoenzymes which appears to be the same activities or constants as the "sensitivity coefficients" of the present invention.

Applicants respectfully submit that Chemnitius *et al.* discloses using a single inhibitor, mipafox, in a single tissue (heart) in a single species (porcine). There is no evidence presented that cholinesterases in other tissues or from other species will behave identically. (In fact, from our assay and the literature, this is not the case.) In Chemnitius *et al.*, since a single inhibitor is

used, the activity of the different cholinesterases is determined by subtraction, i.e. activity differences. Indeed, Chemnitius *et al.* state that one component, atypical ChE, differs from cholinesterase activities of vertebrate tissue and could be identified exclusively in heart muscle, thereby implying that the technique is suitable for assaying amounts in heart tissue, but not as a universal assay for proteins (enzymes) in any samples.

Chemnitius *et al.* calculates activity by subtraction of values determined with one inhibitor at two different concentrations with one substrate. In contrast, the method of the present invention utilizes inhibitors to generate coefficients for any protein regardless of the source, and then uses different substrates to characterize the protein by an additive process. It is important to note that the method of the present invention requires that the sensitivity coefficients are determined once.

The method reported by Chemnitius *et al.*, like most prior art methods known in the art, uses an inhibitor to specifically knock out or partially knock out the activity of the protein of interest, e.g. cholinesterase, and does not address or involve any other proteins or enzymes that may be present in the sample being tested. These types of prior art methods are extremely time-consuming and sample intensive. For example, the prior art methods typically require about 40 to about 45 different concentrations of the inhibitor in order to generate sufficient reliable data that can be used to predict the quantity or activity of each isozyme. A complex mathematical curve fitting routine (rather than simple linear analysis) is required and fraught with error and artifacts.

This can be visualized in Figure 1 of Chemnitius *et al.*, where almost 40 data points are required to fit the experimental points using a weighted least-squares nonlinear curve fitting on the basis of a 3-component model. Due to the resolution of the curve, it is not easily determined if there are even additional components. The method disclosed by Chemnitius *et al.* also used physostigmine, which is covalently bound during the assay, to characterize their cholinesterases. Thus, Chemnitius *et al.* report in table 5 the inhibition of heart muscle cholinesterase by the inhibitor pyridostigmine after mipafox differential inhibition, i.e., a second inhibitor is used on top of enzyme already inhibited. Note that pyridostigmine inhibition alone is complex because it binds and modifies the enzyme and it is well known that this class of inhibitors decarbamylates at some time-dependent rate. This also contributes to inaccuracy unless the turn-over of the inhibitor is known. It is worth emphasizing again that the sensitivity coefficients taught in this

application are derived from the enzyme which is active and not inhibited, while Chemnitius defines his cholinesterases, and a different heart isozyme, by the sensitivity to an inhibitor, and does not define coefficients.

Since Chemnitius *et al.* does not teach or disclose use of the sensitivity coefficients as provided by the present invention, Applicants respectfully submit that the present claims are not anticipated by Chemnitius *et al.* Therefore, the rejection under 35 U.S.C. 102(b) should properly be withdrawn.

Again, Applicants respectfully submit that the cited prior art alone and in combination do not teach or disclose using or calculating standard and universal "sensitivity coefficients" as provided by the present invention in order to assay the activity or concentration of a protein in a sample without isolating the protein from the sample or requiring the use of multiple yet isolated binding reagents.

Rejection under 35 U.S.C. 103(a)

The Examiner rejected claims 40-43 under 35 U.S.C. 103(a) as being unpatentable over U.S. Patent No. 5,674,688 ('688 patent). Specifically, the Examiner deemed that chromatogenic substrates are well known in the art and that one of ordinary skill in the art would have been motivated to select a chromatogenic substrate which is useful for the specific enzyme activities to be monitored.

As explained above, Applicants respectfully submit that determining the sensitivity coefficients and using the sensitivity coefficients as provided by the present invention are not taught or suggested by the prior art. Since the claim 1 is novel and nonobvious and claims 40-43 are dependent thereon, claims 40-43 are also novel and nonobvious. Therefore, the rejection under 35 U.S.C. 103(a) should properly be withdrawn.

Request for an Interview

Should there by any remaining issues after entry of the amendment and consideration of the remarks herein, Applicants respectfully request either an in-person interview or a telephonic interview with the Examiner.

Extension of Time

A Petition for an Extension of Time for two (2) month under 37 C.F.R 1.136 and the appropriate fee are submitted herewith to extend the time for responding to the Official Action to 20 August 2003.

CONCLUSION

All of the stated grounds of objection and rejection have been properly traversed, accommodated, or rendered moot. Applicants therefore respectfully request that the Examiner reconsider all presently outstanding objections and rejections and that they be withdrawn. It is believed that a full and complete response has been made to the outstanding Office Action and, as such, the present application is in condition for allowance. If the Examiner believes, for any reason, that personal communication will expedite prosecution of this application, the Examiner is invited to telephone the undersigned at the number provided.

It is not believed that extensions of time are required, beyond those that may otherwise be provided for in accompanying documents. However, in the event that additional extensions of time are necessary to prevent abandonment of this application, then such extensions of time are hereby petitioned under 37 C.F.R. §1.136(a), and any fees required therefor are hereby authorized to be charged to our Deposit Account No. **210380**, referencing Attorney Docket No. **P66820US1 (00-23).**

Respectfully submitted,
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